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Social Transmission of Food Preferences in a Wild Population of *Mus spretus*

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Para as duas pessoas que mais admiro:

a minha mãe e a minha avó

"Everything is theoretically impossible,

until it is done."

- Robert A. Heinlein

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RESUMO

A informação social, obtida a partir do comportamento, traços fenotípicos e condição de outros indivíduos, pode desempenhar um papel importante na tomada de decisões dos animais. Uma vez que os animais têm exigências ecológicas semelhantes às dos seus conspecíficos, observá-los nas suas atividades diárias, nomeadamente no seu desempenho, e adotar uma estratégia de aprendizagem social poderá revelar-se economicamente mais vantajoso para um indivíduo do que depender unicamente da sua avaliação individual de cada variável ambiental.

Particularmente, quando se trata de decidir o que comer, o papel da informação social pode ser especialmente importante, uma vez que aprender por tentativa-e-erro pode ser um processo moroso e energeticamente custoso, além de poder revelar-se mais perigoso ou até mesmo fatal comparativamente a decisões erradas na escolha de habitats ou parceiros sexuais. Desta forma, a informação obtida inadvertidamente a partir de pistas fornecidas por conspecíficos torna-se uma estratégia adaptativa em indivíduos que carecem de informações pessoais sobre o seu ambiente, mas também quando confrontados com novos ambientes ou desafios.

A transmissão de informação social pode ser categorizada em três tipos: 1) transmissão horizontal, quando ocorre entre indivíduos da mesma geração; 2) transmissão vertical, quando a informação é transferida de progenitor(s) para as crias; 3) ou transmissão oblíqua, quando a informação é transmitida entre um adulto e um juvenil, não diretamente relacionados.

Uma vez que os animais podem aprender uns com os outros o que comer, levanta-se então a questão: poderá a aprendizagem social ser usada na conservação de espécies ameaçadas através da alteração dos seus hábitos alimentares?

Aprendizagem individual através de aversão condicionada a um alimento é provavelmente o método mais utilizado para alterar os hábitos alimentares dos animais no âmbito de programas de conservação, nomeadamente quando espécies invasoras tóxicas passam a ser consumidas pelas espécies locais, com consequências nefastas paras estas últimas. No entanto, embora este método possa criar aversões fortes, tem sido de difícil aplicação *in situ*, uma vez que através da facilitação social – ou seja, através da aprendizagem social que resulta da observação de conspecíficos não condicionados a comerem o alimento associado com a aversão – as aversões condicionadas podem rapidamente extinguir-se na população, exigindo, portanto, uma gestão constante. Uma potencial solução para este problema seria fazer uso da facilitação social logo desde o início.

Vários estudos usando facilitação social na transmissão de preferências alimentares em ratazanas (*Rattus norvegicus*, Berkenhout 1769 e *R. rattus*, Linnaeus 1758) e ratinho-caseiro (*Mus musculus*, Linnaeus 1758) de laboratório têm revelado que a informação social tem uma grande influência nos hábitos alimentares destas espécies, ambas modelando as suas preferências alimentares pelas escolhas dos seus conspecíficos. De facto, no caso dos roedores, uma das formas mais importantes de aquisição de informação social é através de pistas químicas contidas na urina, fezes e durante interações focinho-focinho (por exemplo, através do hálito ou saliva).

Estes estudos têm, sem dúvida, contribuído muito para o conhecimento atual sobre este assunto, uma vez que experiências sob condições laboratoriais controladas são a melhor forma de compreender os processos envolvidos na transmissão social. No entanto, ao se usar exclusivamente linhagens de animais de laboratório para compreender estes processos corre-se o risco de obter resultados com pouca validade comportamental e ecológica, uma vez que a transmissão social entre indivíduos selvagens a

viverem em condições naturais está sujeita a variáveis dificilmente simuláveis em laboratório. Assim, o grande desafio é compreender se, e de que forma, os animais selvagens são igualmente influenciados pelas preferências alimentares dos seus conspecíficos quando expostos a novos alimentos. Só através desta abordagem é que será possível saber se a aprendizagem social tem suficiente relevância em animais selvagens, de modo a posteriormente se poder utilizar esta capacidade em programas de conservação *in situ*.

Como espécie modelo escolhemos usar o ratinho-ruivo (*Mus spretus*, Lataste 1883). A escolha desta espécie teve por base: (1) o facto de nunca ter sido testada transmissão social nesta espécie; (2) ser uma espécie selvagem e bastante comum em habitats naturais; (3) e ser um parente próximo do ratinho-caseiro (*Mus musculus*), uma espécie em que a aprendizagem social de preferências alimentares já foi demonstrada em linhagens de laboratório.

A primeira fase do projeto consistiu em capturar os indivíduos, passando estes imediatamente por uma fase de habituação às condições de laboratório. Seguidamente, foi avaliada a possível existência de uma preferência inata por canela e cacau (teste controlo), as duas substâncias escolhidas como estímulo químico nas experiências de transmissão social seguintes. Tendo como base o paradigma experimental de Galef e Wigmore (1983) e de Valsecchi e Galef (1989), desenvolvemos duas experiências: Experiência 1, um teste de transmissão horizontal, para determinar se a preferência por um novo alimento é transmitida através da interação social de um indivíduo ingénuo (observador) com um conspecífico (demonstrador) que comeu previamente aquele alimento; e Experiência 2, um teste de transmissão vertical, para determinar se a preferência por um novo alimento é transmitida da progenitora para as suas crias. Como alimento novo foi oferecido aos indivíduos ração para roedores polvilhada com canela (RCanela) e com cacau (RCacau), tendo sido os demonstradores condicionados para RCanela e os observadores testados para a quantidade de RCanela consumida face a RCacau.

Nos indivíduos que adquiriram uma preferência por RCanela, foi ainda avaliada a manutenção da preferência adquirida após um período de 30 dias sem contacto com esse alimento. Por fim, determinou-se a eficiência da metodologia usada na transmissão horizontal, sob a forma de uma taxa de sucesso para diferentes graus de preferência alimentar induzida.

Relativamente ao teste de transmissão horizontal, os resultados obtidos indicam que quando os observadores interagiram durante um curto período de tempo (<6s por contato), não adquiriram uma preferência pelo alimento ingerido pelo demonstrador (RCanela), comendo igualmente ambos os alimentos (RCanela e RCacau), tal como os indivíduos do teste controlo. Pelo contrário, quando a interação foi mais longa (>6s por contacto), os observadores demonstraram uma preferência pelo alimento ingerido pelo demonstrador (RCanela). Assim, à medida que a duração das interações focinho-focinho entre indivíduos aumentou, aumentou também a quantidade de RCanela consumida pelos observadores. Isto indica que a transmissão horizontal ocorre em ratinhos-ruivos, contudo, é necessário um tempo mínimo de interação por contacto focinho-focinho, para que a transferência de informação possa ocorrer com sucesso.

Utilizando apenas os indivíduos que tinham adquirido uma preferência durante o teste de transmissão horizontal, os resultados mostram também que os indivíduos mantiveram a preferência adquirida após um período de 30 dias sem contato com RCanela; contudo, observou-se uma diminuição de preferência por RCanela no tempo, sugerindo que interações sociais mais frequentes, bem como o contacto repetido com o estímulo que está a ser transmitido, poderão ser importantes na manutenção de preferência nesta espécie.

Em contrapartida, os resultados obtidos no teste de transmissão vertical sugerem que este mecanismo não está presente em *M. spretus*. No entanto, dado o pequeno tamanho da amostra (4 fêmeas, 1 ninhada cada), não é possível tirar quaisquer conclusões definitivas. Estudos futuros, usando um maior número de indivíduos são necessários para esclarecer se transmissão vertical ocorre ou não no ratinho-ruivo.

Por último, calculámos a eficiência da metodologia desenvolvida, verificando-se que independentemente do grau de preferência considerado (preferência: baixa, >60% RCanela; moderada, >70% RCanela; e alta, >80% RCanela), a percentagem de indivíduos que adquiriram uma preferência pela RCanela foi sempre superior a 50% (58-67%). Consideramos estes valores de eficiência obtidos um bom resultado, especialmente considerando que os animais usados eram provenientes de uma população selvagem, com histórias individuais desconhecidas que poderão ter afetado a propensão dos animais para utilizarem essa informação.

Um dos problemas de muitos métodos de conservação é o seu efeito apenas se fazer sentir ao fim de algumas gerações e, nesse sentido, a sua implementação levar muito tempo antes de se tornar eficiente. Através da transmissão de informação e aprendizagem social, um comportamento pode espalhar-se naturalmente entre os indivíduos de uma população e mais rapidamente do que através de herança genética, podendo, portanto, permanecer conservado numa população durante muitas gerações. Utilizar esta habilidade dos animais em programas de conservação parece, pois, uma boa abordagem, com a vantagem de apenas ser necessário condicionar em laboratório alguns indivíduos da população, que depois de libertados serão os difusores iniciais da informação.

Deste modo, esta metodologia poderá ser transformada numa ferramenta de gestão na proteção e recuperação de espécies ameaçadas que usam a transmissão social de preferências alimentares. É, no entanto, imperativo sempre avaliar cuidadosamente as implicações ecológicas de influenciar e potencialmente mudar os hábitos alimentares de uma população.

Em suma, podemos afirmar que o ratinho-ruivo consegue transmitir informação sobre o alimento recém ingerido através da interação com os seus conspecíficos. A preferência adquirida é mantida ao longo do tempo, embora pareça diminuir ao fim de 30 dias sem contacto com a mesma. Esperamos que os nossos resultados ajudem a reduzir a lacuna existente entre as ações de conservação e o estudo académico do comportamento animal, através da sua inclusão nas medidas existentes, assim como na criação de metodologias adicionais na recuperação de espécies ameaçadas.

PALAVRAS-CHAVE:

Informação social, aprendizagem social, transmissão de preferências alimentares, ratinho-ruivo, ferramenta de conservação.

ABSTRACT

Social information plays a major role in many animal decision-making processes. When it comes to food choices, individual learning via a trial-and-error strategy can end up being deadly, making the acquisition of food related information through social learning an important alternative. Using an experimental paradigm of social transmission of food preferences with an already validated methodology performed in laboratory rats and house mice, we developed an experiment with wild Algerian mice (*Mus spretus*, Lataste 1883), to understand whether wild animals are similarly influenced by their conspecifics' food choices.

By developing a horizontal transmission test, we were able to induce preference for one of two novel foods (cinnamon and cocoa flavored chows, CCinnamon and CCocoa) in naïve individuals after social interaction with a conspecific that had previously eaten CCinnamon. However, the transmission of information depended on the duration of nose-to-nose contact between individuals, since only when individuals interacted for a minimum average time of ≈6 seconds did they acquire a preference for CCinnamon. Also, individuals that acquired a food preference were able to maintain it over a 30-day period in the absence of additional contact with CCinnamon. On the other hand, food cues were not successfully transmitted between *M. spretus* females and their offspring, as juveniles did not show a preference for CCinnamon relative to CCocoa. However, considering the small sample size used (N=4 females, 1 litter each) we cannot draw any definitive conclusions.

Lastly, for the horizontal transmission test, we also calculated the efficiency of the methodology used. Independently of the preference criteria adopted (preference: low, >60% of CCinnamon; medium, >70% of CCinnamon; and high, >80% of CCinnamon), the percentage of individuals that acquired a preference by CCinnamon was always superior to 50% (58-67%). We consider the efficiency values obtained as good results, especially considering that the animals used came from a wild population and had and unknown background of social influences that may have affected the propensity of the animals to use that information.

Given the exponential progression of social learning through a population, using social transmission in conservation programs to induce a change in behavior might often represent a reduction in terms of both costs and time. Besides, only a few individuals need to be induced to change their feeding habits for it to rapidly spread in the population. Therefore, we strongly believe that the transmission of food preferences in wild *M. spretus* should serve as an indication that this approach should be taken outside the laboratory and used as a new management tool in species conservation.

KEYWORDS:

Social information, social learning, food preference transmission, Algerian mice, conservation tool.

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ABBREVIATIONS

AverageIntTime	Average Interaction Time
CCinnamon	Cinnamon flavored chow
CCocoa	Cocoa flavored chow
Dem	Demonstrator
DifWeightDemObs	Weight difference between Demonstrator and Observer
IntervalsTimeGroup	Interval groups/classes between average interaction times
IntervalsTimeGroupAndControl	Interval groups/classes between average interaction times plus the control individuals
MaxIntTime	Maximum Interaction Time
NumInt	Number of Interactions
Obs	Observer
ProportionCCin	Proportion of cinnamon flavored chow
SameDem	Same Demonstrator
SexInd	Sex of the Individuals
SexObs	Sex of the Observer
SexOff	Sex of the Offspring
TotalIntTime	Total Interaction Time

1. INTRODUCTION

TRANSMISSION OF SOCIAL INFORMATION

Social information, the information acquired from the behaviors, phenotypic traits and condition of other individuals (Danchin *et al*. 2008; Wagner and Danchin 2010), can play an important role in shaping animal decisions. Evidence for the transmission of social information between animals has been shown in many different taxonomic groups and circumstances. Some of the best known examples are the feeding tool traditions used by different chimpanzee populations (Whiten *et al*. 1999); the learned ability of some black rat populations to strip pine cones (Aisner and Terkel 1992; for review see Terkel 1996); the choice of breeding patches by collared flycatchers (Doligez *et al*. 2002); the cultural diffusion of a technique to open a puzzle feeder door by great tits (Alpin *et al*. 2015); and the mate preference copying of guppies (Auld and Godin 2015) and *Drosophila,* Fallén 1823 (Mery and Varela *et al*. 2009) after observing conspecific mating decisions.

Because animals share similar ecological requirements with their conspecifics, observing their performance in their daily activities could help any one individual, depending on their personal experience, in deciding whether to stay or leave from a given habitat patch, whether to eat or avoid a particular food item, or whether to accept or reject a certain mating partner. Such a strategy can be seen as an economical short-cut to the decision making process, compared to the individual assessment of every environmental variable (Danchin *et al*. 2004, 2008).

Particularly, when it comes to deciding what to eat, the role of social information may be especially important, as learning through trial-and-error (experience-based learning) is both time and energy consuming and may also prove more dangerous or even deadly than erroneous decisions regarding habitats and mates. Also, experience-based learning does not imply that animals have better adaptive choices. A study performed in omnivorous harvestmen spiders (*Heteromitobates discolor*, Sundevall 1833) showed that an individual can learn to eat a specific food through individual experience that can turn to be a maladaptive choice, decreasing his own longevity (Costa *et al*. 2016).

Therefore, by acquiring information from conspecifics' feeding habits, animals can be a step ahead to make the best decisions about the food they eat and reduce the need for trial-and-error learning (Danchin *et al*. 2004; Kendal *et al*. 2009). Because social cues are transmitted inadvertently by individuals (inadvertent social information; Danchin *et al*. 2004, 2008; Wagner and Danchin 2010) they are not signals under selection to covey information. This means that such cues cannot be faked, and are thus highly reliable sources of information (Danchin *et al*. 2008). Consequently, if the information acquired about a new food source is provided by healthy or unhealthy conspecifics, it reliably reveals its safety and/or quality (Kavaliers *et al*. 2005; Boillat *et al*. 2015).

A consequence of social information use by animals is the long-lasting transmission of behavioral traditions in a process similar to cultural inheritance in humans. Scientists thus started to recognize the existence of culture in non-human animals. Culture can be defined as non-genetic but heritable behavioral traits shared by a group of individuals that is passed among them through social transmission and social learning (Danchin *et al*. 2004; Laland and Janik 2006; Danchin 2013). Although the existence of non-human animal cultures is still an ongoing debate (Laland and Janik 2006), it is now widely accepted that the inheritance of non-genetic information affects variation and selection, and thus the evolution of species (Danchin *et al*. 2004, 2011; Laland *et al*. 2015). By changing behavioral strategies and creating niches with distinct behavioral traditions, the transmission of social information gained an

important role in the way we understand evolution (Danchin 2013; Laland *et al*. 2015), increasing the importance of studying animal communication and social learning.

Following Cavalli-Sforza and Feldman (1981) cultural inheritance, or the transmission of social information, can occur in three different ways: 1) horizontal transmission, when it occurs between conspecifics from the same age class or generation; 2) vertical transmission, when the information is transferred between parent(s) and offspring; 3) and oblique transmission, when the information is transmitted between a non-directly related adult and a young conspecific. When the information is transmitted vertically, it generally causes longer-lasting effects in a population's behavioral traditions, like, for instance, traditions about food preferences, with consequences in terms of the selective pressures exerted over the preyed foods. When the information is transmitted obliquely or horizontally, it may not persist in a population for many generations, but it will spread rapidly among individuals in only one generation, which may still exert significant selective pressures in the environment (Danchin and Wagner 2010; Danchin *et al*. 2011).

Nonetheless, social learning can only be considered an adaptive strategy if individuals lack personal valuable information about their environment. This generally occurs in young (inexperienced) individuals, but also in adults when facing new environments or new challenges (Valone and Templeton 2002; Laland 2004; Wagner and Danchin 2010), or when an individual's personal information leads to maladaptive decisions (Costa *et al*. 2016). If the majority of individuals in a population depended on social information, animals would only copy each other instead of gathering information directly from the environment (Laland 2004). In time, this would eventually lead to erroneous, instable informational cascades that could break in a relatively short period (Giraleau and Valone 2002). For instance, bumblebees make their foraging decisions based on three parameters: innate biases, previous individual experience and social information acquired from observing conspecifics' foraging behaviors. When they find a food source of good quality, they repeatedly visit that same site based on their personal information. Only when the food source becomes depleted and they lack information about a new one, or the food source is of an unknown type, will they learn from their nest mates where to forage next, or whether the food is safe or not. Hence, bumblebees use social information but only dependently of each individual's personal experience (Jones *et al*. 2015).

SOCIAL FOOD CONDITIONING IN CONSERVATION ACTIONS

Individual learning through conditioned food aversion (Garcia *et al*. 1974) is probably the most widely used method to change animals' food habits within conservation schemes (Gustavson and Gustavson 1985; Ralphs and Provenza 1999; O'Donnel *et al*. 2010). It consists on creating an association between the ingestion of a particular food item and gastrointestinal distress, so that animals will avoid that food in the future. However, although this method can create strong aversions, it has been a difficult method to implement in the field, since through social facilitation (by seeing non-conditioned conspecifics eating that particular food), the conditioned aversions can rapidly extinguish in the population (Gustavson and Gustavson 1985; Galef 1986, 1989, 1996; Ralphs *et al*. 1997; Ralphs *et al*. 1994; Ralphs and Provenza 1999), thus requiring constant management (O'Donnel *et al*. 2010).

Nonetheless, food aversion conditioning has been successfully used in conservation actions. An especially interesting study is that made with the northern quoll (*Dasyurus hallucatus,* Gould 1842), a small, cat-sized Australian carnivorous marsupial. One of the major threats to the northern quoll is the presence in its habitat of the highly toxic cane toad (*Rhinella marinus,* Linnaeus 1758), which is an invasive species that the quoll recently introduced in its diet. The outcome of preying on this species is quolls' death following severe gastrointestinal inflammation and cardiac failure. The impact to quolls' populations was so big, that quolls were progressively becoming locally extinct following the toads'

invasion across Northern Australia. By feeding captive quolls ("toad-smart") with small non-lethal dead toads (juvenile toads lack the adults' large deadly parotoid glands), together with a nausea-inducing but otherwise harmless chemical, the authors were able to induce an aversion toward cane toads, and consequently a decrease in the quolls' attacks directed towards this species, increasing the survival rate of the trained animals (O'Donnel *et al*. 2010). The same procedure was successfully tested in Australian bluetongue lizards (*Tiliqua scincoides,* Gray 1825) whom, like quolls, also needed protection from deadly encounters with cane toads (Price-Rees *et al*. 2011). However, for the implementation of such a solution at a large scale, the authors of both studies suggest that constant intervention in the field, by aerially deploying taste aversion baits in quolls' and bluetongue lizards' habitats is necessary, a solution which is both expensive and difficult to implement.

An alternative solution is to make use of animals' social learning abilities, as in the elucidative example of great tits (*Parus major,* Linnaeus 1758). A few individuals (demonstrators) were caught in the wild (Wytham Woods, UK) and trained in the laboratory to open a puzzle box feeder by either opening a blue or a red door. The trained demonstrators were then released in their correspondent sub-populations and puzzle box feeders were installed in each habitat patch. From only 2 trained individuals *per* sub-population, conspecifics were heavily biased towards opening the feeder using the same door color originally learned by the demonstrators released in their sub-population. This behavior was maintained for at least 2 generations and the individuals that migrated between patches copied, by social facilitation, the local tradition instead of using the technique of their original population (Alpin *et al*. 2015).

Besides great tits, some of the best-known studies of transmission of food preferences by social facilitation were done in laboratory rats (*Rattus norvegicus* Berkenhout 1769 and *R. rattus*, Linnaeus 1758). These studies showed that preference for a novel food was transmitted through social nose-to-nose interaction with a conspecific that had previously eaten that same food (Galef and Wigmore 1983; Posadas-Andrews and Roper 1983). The acquired preferences were also maintained for at least 30 days after the social interaction, suggesting that a long-term memory process is probably involved in the social acquisition of food preferences (Galef and Whiskin 2003). Additionally, it was shown that the food's chemical cues can also be passed to rat pups during pregnancy through the placenta (Hepper 1988), the mother's milk during nursing (Galef 1977; Galef and Henderson 1972; Martin and Alberts 1979), the odor carried by parents, as well as by the environment where they grow (Galef 1971, 1981; Galef and Clark 1971a,b), thus conditioning individuals, from a very early age, on which foods to eat.

Similar experiments have been carried with laboratory house mice (Valsecchi and Galef 1989) and reached similar conclusions: individuals seem to model their food preferences by those of their conspecifics.

Altogether these studies demonstrate that if social facilitation can extinguish conditioned food aversions gained by individual learning, it also can, on the other hand and very effectively, help to disseminate, by social learning, new techniques to obtain food and new food preferences. To our knowledge, animals' social learning abilities have never been used in conservation actions. However, recent developments in the northern quoll conservation project suggest that it can clearly be used as an effective conservation tool. After re-introduction, "toad-smart" females seem to have naturally transmitted cane toad aversion to their offspring in what it seems to be a process of social facilitation, which in this case reinforced the aversion instead of extinguishing it; cane toad avoidance is now naturally spreading through the population most probably by the offspring watching their mothers sniffing and rejecting them as food, preferring to feed on other prey (Webb *et al*. 2015).

CHOOSING THE MODEL SPECIES

Although a plethora of studies has been done in social learning of food preferences (for review see Galef 1996; Terkel 1996), most have used laboratory animals due to logistical or ethical reasons (Pesendorfer *et al*. 2009). By laboratory animals we refer to an animal that is cared, fed and bred by humans, with domestication traits being selected over many generations (Price 1984; Kunzl *et al*. 2003). These studies have undoubtedly contributed much to the current knowledge on this subject, since controlled conditions are often necessary to better understand the processes behind such mechanisms (Kendal *et al*. 2010). However, using long-term lineages of laboratory animals may make the results more difficult to interpret, potentially lacking ecological and behavioral validity, since social transmission in wild populations could be constrained by variables not accounted as easily in laboratory studies, such as dominance hierarchies, territorial behaviors, and presence of predators, among others (Kendal *et al*. 2010).

Studying social learning with wild animal populations, both in the laboratory and in their natural habitats, is thus one of the great challenges ahead. Indeed, the number of social learning studies done with wild animals under natural conditions, such as the great tit study described above (Aplin *et al.* 2015), or the studies on magpie-jays (Langen 1996), and meerkats (Thornton and Malapert 2009) are increasing (for a review see Galef 2004; Reader and Biro 2010 and Kendal *et al*. 2010).

Rodents acquire most information from conspecifics through chemical cues contained in urine, feces and during nose-to-nose interactions (e.g., through breath, sniffing and licking). Chemical cues contain information about an individual's identity, sex, age, reproductive state and social status, but also about recently eaten food and health condition (Eisenberg and Kleiman 1972; Wyatt 2003; Kavaliers *et al*. 2005). Socially transmitted chemical cues can be especially important when making choices, and in some instances even overcome previous preferences; female house mice always prefer to mate with males that are uninfected with gastrointestinal nematode parasites, however, they attenuated the aversion for infected males when they were associated with the odor of another estrous female (Kavaliers *et al*. 2006). In terms of transmission of food preferences, carbon disulfide (CS_2), one of the main components of mice breath volatiles, is responsible, at least in part, for the transmission of information between individuals (Galef *et al*. 1988; Bean *et al*. 1988; Munger *et al*. 2010). When rats are exposed to a novel food in the presence of carbon disulfide, they exhibit a subsequent preference for that food, but no preference was observed when rats were exposed to the same food alone (Galef *et al*. 1988).

The Algerian mouse, *Mus spretus* Lataste 1883 (figure 1.1), is a Mediterranean endemic species, occurring in Portugal, Spain (excluding the northern fringe), southern France and Northern Africa (Morocco, Algeria, Tunisia and Libya. *M. spretus* is fairly abundant in Portugal (Mathias 1999), having a "Least Concern" conservation status according with the IUCN list of endangered species. It lives preferentially in grassland, dry shrubland and open woodland. Although it is not a commensal species, it can occasionally be found in abandoned buildings and a variety of agrosystems (Palomo *et al*. 2009). Being mainly nocturnal, its main peaks of activity occur at dusk and dawn, though these can change seasonally (Vargas *et al*. 1987). In terms of diet, *M. spretus* is an opportunistic omnivore, mainly feeding on grass seeds, fruits and small insect larvae (Palomo *et al.* 2009). The species is polygamous: males establish a dominance relationship and defend non-overlapping territories that may include several female nests. Territories are identified by the use of scent marks deposited by males but they are relatively tolerant to intruders (Hurst *et al*. 1996, 1997).

Figure 1.1 - Algerian mouse, ***Mus spretus*, Lataste 1883**

Similarly to other rodents, the Algerian mouse relies significantly on its olfactory system to acquire information about its surroundings, food and conspecifics (Hurst *et al*. 1996, 1997). Furthermore, being a close relative of the house mouse (*Mus musculus,* Linnaeus 1758), a species where social transmission of information has already been shown to exist (in laboratory animals only, Valsecchi and Galef 1989), the same validated behavioral paradigms should be easily implemented in the Algerian mouse. Moreover, the Algerian mouse is a fairly abundant species, easy to live-trap, and also known to adjust well to laboratory conditions. Hence, by providing the opportunity to combine a laboratory study with the use of wild individuals, the Algerian mouse, is the ideal model for this study.

OBJECTIVES, HYPOTHESES AND PREDICTIONS

Taking into consideration the ways by which animals acquire and use social information could indeed be key to the success of conservation programs (Blanchet *et al*. 2010). If when lacking valuable personal information animals can learn from each other where to eat – as well as where to live (e.g. Doligez *et al*. 2002) and with whom to mate (e.g. Mery & Varela *et al*. 2009) –, and if individual preferences can sometimes be overridden by social learning, the question of if and how social learning could be actively used in conservation actions arises. With this study we ask how social food conditioning can be implemented to change animals' food habits and use this mechanism to help in the recovery of endangered species.

In particular, with this project we aimed to understand if preference for one of two novel foods is socially transmitted in the Algerian mouse. By preference we mean the food choice made by the tested subjects when facing two options. Following the methodological approach proposed by Kendal *et al*. (2010) on how to implement social learning research outside the laboratory, we first wanted to determine whether social transmission of food preferences at the individual level – between dyads of experienced and naïve individuals – occurs in the Algerian mouse under controlled laboratorial conditions. Only then, in a second phase of the project, can the social diffusion of new food preferences be tested at the population level in the wild. My master thesis consisted of the first phase.

Two modes of social transmission were considered: a) horizontal social transmission, to test if the preference for one of two novel foods could be induced through the social interaction of a naïve adult individual with an adult conspecific that had previously eaten one of those foods; and b) vertical social transmission, to test if preference for one of two novel foods was transmitted from mother to its offspring during pregnancy and through milk and/or other chemical cues during nursing. Moreover, we also aimed to determine whether the naïve individuals maintained their socially learned food preference even if deprived of the new food for a long period of time (30 days).

The experiments developed in this thesis were based on Galef and Wigmore's (1983) and Valsecchi and Galef's (1989) validated experimental paradigm with laboratory rats and house mice, respectively. Hence, for comparison purposes, and despite a few necessary changes to the experimental protocol, we

used the same food flavors of these studies, which means that the novel foods that were provided to our tested subjects were rodent chow flavored with either cocoa or cinnamon powder (more details in the Methods section).

Given that *M. spretus* is a generalist in terms of diet, individuals are probably predisposed to try novel foods and are thus more likely to eat unpalatable and potentially toxic food items. Hence, the use of social information as part of the species foraging strategy may be especially important both as a way to save time and energy trying to learn about novel foods independently, but also to avoid costly choices (Galef and Giraldeuau 2001). We therefore predicted that, similarly to rats (Galef and Wigmore 1983) and house mice (Valsecchi and Galef 1989), *M. spretus* should also horizontally transmit food preferences to their conspecifics through social interactions.

Regarding vertical transmission, learning from one's mother experience with food should improve the performance of food finding by young mice in the wild and should keep them safe from inadequate foods for quite some time. Maternal effects are, indeed, known to be extremely important to the offspring's adaptation to local conditions and constitutes the most effective and long-lasting way of social information transmission (Danchin *et al*. 2011). Hence, we predicted that the offspring should acquire a strong food preference for what their mothers ate.

Regarding long-term effects, since some foods consumed by *M. spretus* are seasonal, not being available in the wild permanently, it is expected that this species memorizes which foods are safe for long periods of time. Moreover, as laboratory rats seem to have long-term memory and memorize food odors learned from their conspecifics (Galef and Whiskin 2003), our third and final hypothesis was that wild *M. spretus* should also have this ability. Hence, we predicted that our test subjects should maintain their acquired food preference for at least a 30-day period.

2. METHODS

ANIMAL TRAPPING, MAINTENANCE AND HABITUATION PERIOD

Specimens of the Algerian mouse, *Mus spretus*, were captured within the natural Park of Sintra-Cascais, between November 2015 and July 2016, using Sherman and wood live traps baited with sardine paste (figure 2.1). Traps were checked every two and a half hours, starting at dusk until 1am.

Figure 2.1 - Live traps. Sherman (left) and wood (right) live traps.

Animals were taken to the small mammal laboratory at the Faculty of Sciences of the University of Lisbon, and maintained under controlled laboratory conditions (20 ± 2ºC; 12h:12h light:dark cycle; lights on at 8:00am). Mice were housed individually in size 2 Makrolon cages (20.5x26.5x13.5cm) or in mating pairs (see experiment 2) in size 3 Makrolon cages (26.5x42.5x14cm). Wood shavings were used as bedding; a cardboard tube, tissue paper and cotton were offered as nesting material and environmental enrichment. Food (rodent chow safe04) and water were provided *ad libitum*. All animals were weighed at arrival and submitted to a habituation period of 4-8 days to the laboratory conditions. Mice weight was monitored on a daily basis during the course of the experiments to outwit any problems associated with the experimental protocols and the habituation process to the laboratory.

After the experiments, all animals were released at their respective capture locations (see more details about this in the ethical note, at the end of the methods section).

M. SPRETUS NATURAL PREFERENCE FOR COCOA AND CINNAMON FLAVORED FOODS – CONTROL TEST

The first step of the experimental procedure was a control test, in order to determine whether *M. spretus* had a natural preference for any of the new foods to which they were going to be exposed in the laboratory: cocoa and cinnamon flavored food.

The novel food types were made by flavoring regular mice chow with either 2% of cocoa (Chow Cocoa - “CCocoa”) or 1% of cinnamon powder (Chow Cinnamon – “CCinnamon”). The rational for using cocoa and cinnamon as flavorings was based on the assumption that wild *M. spretus* had never been in contact with neither of the substances. Additionally, both substances have successfully been

used as flavorings in a similar experiment with a validated methodology using laboratory rats (Galef and Wigmore 1983) and house mice (Valsecchi and Galef 1989).

Since *M. spretus* were not expected to have had any previous contact in their natural habitat with cocoa or cinnamon flavored foods, our hypothesis was that they should not show a preference or distaste for either of those flavors in the control test, and should thus eat from both flavored chows equally. The data obtained here provided a baseline with which to compare the preference behavior of mice tested in horizontal and vertical social transmission experiments.

SUBJECTS: 24 Algerian mice were used in this experiment, 16 males and 8 females.

PROCEDURE: During the habituation period, mice were fed with regular rodent chow (Chow Neutral – "CNeutral"). Having completed the habituation period, every mouse was offered ≈10g of CCocoa and ≈10g of CCinnamon. Both foods were placed on opposite sides of the cage; two partitions placed on the cage feeder kept the food 5 cm apart to avoid the mixing of the two chows and subsequent flavor contamination; each food was alternately placed on the right or left side of the cage feeder to control for side bias effects. Subjects and both food types were weighed daily between 10 and 11 am during a 3-day period, and each day the uneaten food was replaced by ≈10g of fresh flavored chows in order to prevent flavor reduction over time. Monitoring the food eaten over a 3-day period allowed to control for the possibility that the mice would show a food preference only in the first day, or that, on the contrary, they only show or gain a preference after the first or second day (figure 2.2).

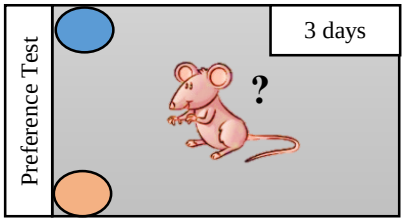

Figure 2.2 - Control preference test set up. *Mus spretus* individuals were offered a choice between CCinnamon (blue) or CCocoa (orange), during a 3-day period.

EXPERIMENT 1 – HORIZONTAL SOCIAL TRANSMISSION

With this experiment we aimed to determine if horizontal transmission of food preference occurs in the Algerian mouse. For that, we tested whether naïve individuals (the observers) acquired a preference for one (CCinnamon) of two novel food types (CCinnamon *versus* CCocoa) after nose-to-nose social interactions with a conspecific (the demonstrator) that had previously eaten that food (CCinnamon). Finally, by performing a long-term preference test, we evaluated whether the food preference, if acquired, would be maintained after a 30-day period during which they had no contact with the novel food type.

If horizontal transmission of food preferences occurs in this species, we predicted that the tested subjects should eat significantly more, compared to the control subjects, from the chow that the demonstrator mice had eaten (CCinnamon) and additionally, that this preference, if acquired, should be maintained after 30 days.

HORIZONTAL TRANSMISSION PREFERENCE TEST

The methodology applied here was based on the experiments from Galef and Wigmore (1983) and from Valsecchi and Galef 1989 (see also Galef and Whiskin 2003), since we wanted to understand if their validated behavioral paradigm for the rat and the house mouse would also apply to the Algerian mouse, and using individuals caught in the wild, with an unknown background of social influences. Yet, because we used a different species some modifications to their experimental protocol where necessary namely: (a) we used male-female dyads, both males and females being used as demonstrators and observers, as opposed to only using males, because in wild populations of Algerian mice male-female dyads are the most common social interactions (Hurst *et al*. 1996, 1997; see more details below); (b) observers and demonstrators were not placed together in the habituation period – which in the other protocols was implemented for familiarization purposes–, since we were testing males with females and did not want them to mate; (c) demonstrators were not deprived of food before testing, because we did not want to induce additional stress to the individuals (they were not reared in the laboratory), which could affect the interaction with the observer; to maximize the probability that the demonstrators had eaten recently, interactions were performed following the individuals' early morning activity peak (reaching a maximum immediately after dawn; Palomo *et al*. 2009). Also, we wanted to try to approximate the laboratory conditions to those of a wild situation, where they probably are not without food for such a long time period; (d) the interactions lasted for 30 minutes instead of 15 minutes, to enhance the probability of nose-to-nose contact, as these animals are territorial and hence may need additional time to gain confidence with one another before starting a social interaction; and (e) the observer was offered the two food types for 72h instead of 60h to allow a direct comparison with the control test.

SUBJECTS: 36 Algerian mice were used in this experiment, 24 as observers, from which 14 were males and 10 were females; and 12 as demonstrators, from which 5 were males and 7 were females.

PROCEDURE: Animals were assigned to two groups at arrival in the laboratory, the demonstrator group (Dem) and the observer group (Obs). During the habituation period, the demonstrators were immediately fed with CCinnamon (to ensure their breath had a strong scent of flavored cinnamon chow when the interaction occurred in the testing phase), while observers were fed with CNeutral. After this period, dyads of one Dem and one Obs were set. However, to decrease the number of mice used per experiment, each demonstrator was used with two different observers, on separate days. In order to determine whether gender influences the transmission of information in *Mus spretus,* both females and males were used as observers and demonstrators, meaning that social interactions were always performed between opposite sex conspecifics (female Dem with male Obs and male Dem with female Obs). Other dyads could have been created, but we opted to first test the occurrence of social transmission in *M. spretus* by using the social interaction that is thought to most frequently occur in a wild setting i.e., female-male. Males are polygamous, defending non-overlapping territories that include several female nests. Therefore, males contact directly more often with females, and vice-versa, than males with other males (scent marks in their territories avoid entrance of neighboring males, so male encounters are rare) or females with other females (as their vital areas are smaller) (Hurst *et al*. 1996, 1997).

The experimental protocol had two phases: the demonstration or social interaction phase and the post-demonstration or preference test phase. Social interactions were performed by placing one Dem and one Obs in a glass terrarium for a 30-minute period. A metal wire mesh placed in the middle of the terrarium prevented mice from direct contact, with the exception of nose-to-nose interactions (e.g., sniffing and licking), while allowing access to each other's chemical cues (Galef and Wigmore 1983; Valsecchi and Galef 1989) (figure 2). Interactions were staged earlier in the day (9 am), a period of

known major activity in mice (Vargas *et al*. 1987; Gray *et al*. 1998) (figure 3). This was especially important to increase the likelihood that the Dem had recently been eating and, consequently, a strong odor stimulus from its breath was present during the interaction with the Obs. Interactions were videotaped (Canon LEGRIA HFM46) and posteriorly analyzed to assess the influence of the number and duration of nose-to-nose interactions in the transmission of information. This was important because Dem and Obs could spend different amounts of time in actual nose-to-nose interactions and hence affect social information transmission. Glass terrariums were washed between social interactions with detergent and water and afterwards with alcohol 70 %, to ensure that odor cues from previous test individuals did not influence subsequent test results.

After the social interaction phase, both Dem and Obs were returned to their cages. Demonstrators were offered CCinnamon *ad libitum*, while Obs initiated the preference test phase and offered ≈10g of CCocoa and ≈10g of CCinnamon. Both foods were available to the mice in the same way as in the control test. Each Obs and both flavored foods were weighed daily for a 3-day period (figure 2.3). As in the control experiment, food was replaced daily, to ensure that a strong flavor was always present.

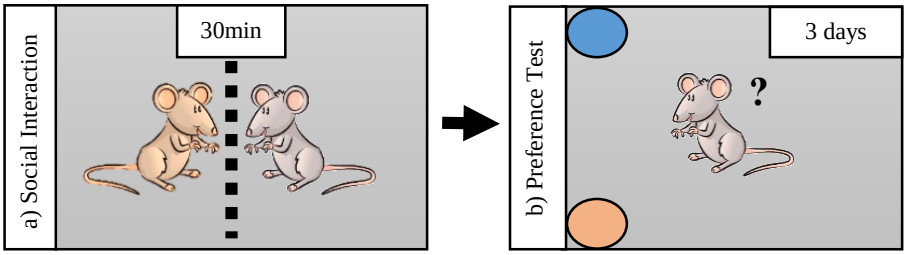

Figure 2.3 - Horizontal Transmission test set up. a) Social interaction between a ***Mus spretus*** demonstrator (left) and a *M. spretus* observer (right), during 30 minutes, separated by a mesh (dashed line); b) Preference test, where observers had a choice between CCinnamon (blue) or CCocoa (orange), during a 3-day period.

LONG-TERM PREFERENCE TEST

In order to determine if individuals maintained their preference for the novel food over time we performed a long-term preference test using the same tested subjects from the horizontal transmission test. Therefore, after the preference test phase of the horizontal transmission test, individuals were maintained on a diet of CNeutral for a 30-day period. After this period, individuals were again offered ≈10g of CCocoa and ≈10g of CCinnamon and their preference was evaluated the same way as in the preference test described above (figure 2.4). Only individuals that showed a preference for CCinnamon were used.

Figure 2.4 - Long-term preference test set up. *Mus spretus* observers that showed a preference for CCinnamon in the horizontal transmission test had a choice between CCinnamon (blue) or CCocoa (orange), 30 days after the social interaction.

EXPERIMENT 2 – VERTICAL SOCIAL TRANSMISSION

PILOT TEST

Although all pregnant females captured during field work were always released immediately at the site of capture, one pregnant female was unintentionally brought to the laboratory to participate in the horizontal transmission test. Once in the laboratory, she was randomly selected as a demonstrator, being fed with CCinamon. After a few days, we noticed she was pregnant when she successfully gave birth to a litter in the laboratory and thus offered the opportunity to perform a pilot test and investigate if preference for a novel food (CCinnamon) was transmitted vertically from mother to offspring through social interactions during the offspring growth phase. Since these individuals, besides being born in the laboratory, were not artificially selected, domestication characteristics (distinctive of a laboratory animal) were not expected to appear, as they need several generations in captivity to develop. Indeed, first generation animals born in the laboratory should maintain the majority of the species' typical behaviors, as well as the same hormonal responses to stress (Künzl *et al*. 2003).

SUBJECTS: 4 mice born from 1 captured pregnant female, 1 male and 3 females.

PROCEDURE: When the pregnant female gave birth, she was housed in a size 3 Makrolon cage with her offspring and was provided with CCinnamon *ad libitum*. This diet was maintained until the offspring were 15 days of age (i.e., weaning age, after which *M. spretus* starts eating solid food; Palomo et al. 2009). From this day on, all animals were fed with non-flavored food (CCNeutral). This ensured that the offspring did not have direct contact with cinnamon flavored food before being tested. At the 30th day of age, each juvenile was housed individually in size 2 Makrolon cages and offered ≈10g of CCocoa and ≈10g of CCinnamon, as described in the horizontal transmission experiment. Each offspring and both flavored foods were weighed daily for a 3-day period (figure 2.5). Again, food was replaced daily, to ensure that a strong flavor was always present.

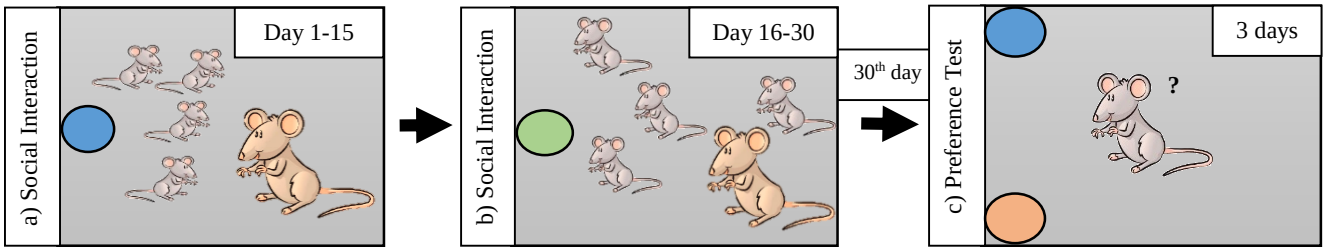

Figure 2.5 - Pilot Vertical Transmission test set up. a) day 1-15: social interaction between a female *Mus spretus* and her offspring where the female ate CCinnamon (blue); b) day 16-30: social interaction between the female and her offspring, where the female and the offspring ate CNeutral (green); c) day 30: offspring preference test at 30 days of age, where they had a choice between CCinnamon (blue) or CCocoa (orange), during a 3-day period.

VERTICAL TRANSMISSION PREFERENCE TEST

After the pilot test, which gave a positive indication that vertical transmission can occur in *M. spretus* (see details in the Results section), we wanted to validate our finding with additional individuals and with the introduction of CCinnamon during pregnancy. Hence, our aim was to formally test whether females could transmit a food preference to their offspring, i.e., if vertical transmission of food preference occurs in the Algerian mouse, not only during nursing, but also during pregnancy, similarly to what occurs in rats (Hepper 1988; Galef 1977; Galef and Henderson 1972; Martin and Alberts 1979; Galef 1971, 1981; Galef and Clark 1971a,b).

SUBJECTS: 5 pairs of mice, previously used in the horizontal transmission experiment, were mated, however, one of them never produced offspring. The pairs were from 3 different locations within the natural park of Sintra/Cascais to minimize the impact of posteriorly introducing new animals in the capture areas (since all animals, captured or born in the lab, were released into the wild after the experiments). Each female had between 2 to 5 offspring (one female had a litter of 7 but two pups died before the 30th day of age and hence before the testing phase). In total 13 offspring were used (6 males and 7 females).

PROCEDURE: Each pair was housed in size 3 Makrolon cages and maintained under controlled laboratory conditions as described above. After mating, the animals were provided with CCinnamon *ad libitum*. After the offspring were born, the same procedure and feeding regime used in the pilot test was applied (described above). Males were removed from the cage immediately after the offspring was born to avoid consecutive mattings (figure 2.6).

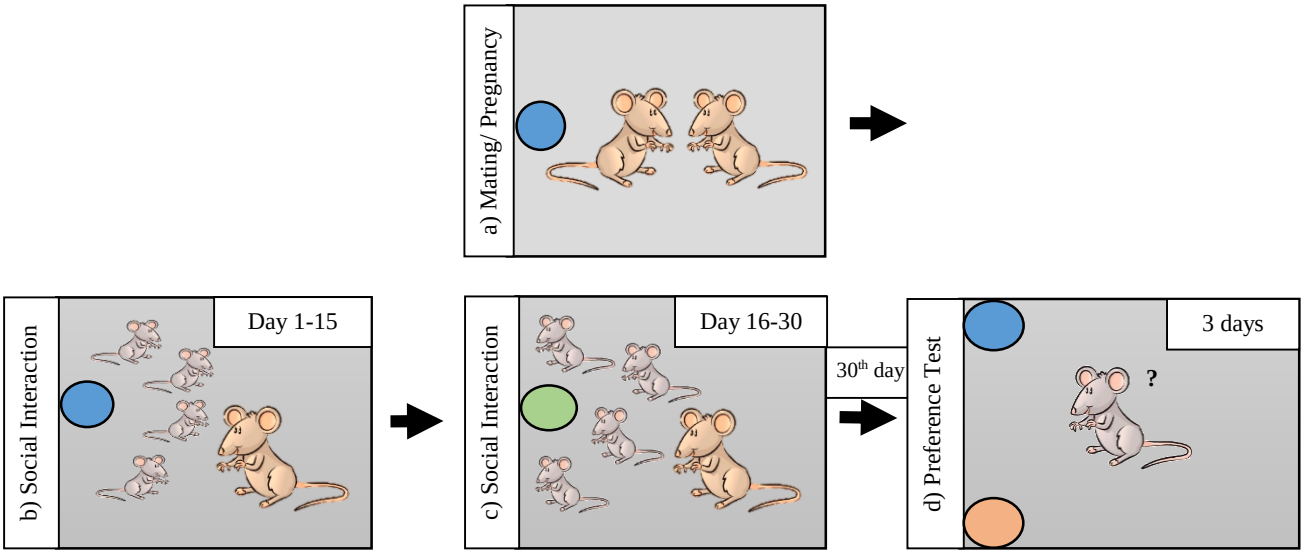

Figure 2.6 - Vertical Transmission test set up. a) Pairs of mice ate CCinnamon, while they were mating and during pregnancy; b) day 1-15: social interaction during 15 days, between mother and her offspring, where the mother ate CCinnamon (blue); c) day 16-30: social interaction, during 15 days, after the offspring, where the mother and the offspring ate CNeutral (green); d) day 30: offspring preference test at 30 days of age, where they had a choice between CCinnamon (blue) or CCocoa (orange), during a 3-day period.

LONG-TERM PREFERENCE TEST

As in the Horizontal Social Transmission Experiment, if individuals gained a food preference for CCinnamon, we tested whether this preference was maintained after a period of 30 days without contact with CCinnamon (described above; figure 2.4).

STATISTICAL ANALYSIS

For each statistical test we used R 3.0.3-main (R Development Core Team 2016) and Excel 2013. The response variable was always the preference for CCinnamon (*ProportionCCin*). It was calculated using the amount of CCinnamon eaten by each individual per day relatively to the total amount of both chows eaten, using weight of chow eaten in grams (g) as the measure unit:

$$\textbf{2.1}\ ProportionCCin = \frac{CCinnamon\ Eaten\ (g)}{Total\ food\ eaten\ (CCocoa+CCinnamon)(g)}$$

To analyze data, we applied a single sample *t*-test, several Linear Mixed Models, and a non-parametric Kruskal-Wallis test. Linear Mixed Models (LMM), fit by restricted maximum likelihood (*lmer* function from the lme4 package), were applied to account for both fixed and random effects (Bates *et al*. 2015). All the assumptions of the *lmer* function, regarding the normality and homocedascity of the residuals, were respected. As the models were being applied, every time a fixed or a random factor was non-significant it was removed by backward stepwise selection, until we ended-up with the best and simplest model we could get (best reduced model). To successively choose the best model between two possible models we used the *anova procedure* (likelihood ratio test, using the Chi-squared test due to the Wilk's Theorem) that comes with the lme4 package. The simplest model was always preferred if the difference between the two models was not significant. Afterwards, for the best reduced LMM models obtained, we always performed the *Anova procedure* (F-test) of the lme4 package (Bates *et al*. 2015) to understand if the variables (although included in the model) had a significant effect in the proportion of cinnamon flavored food that was eaten by the tested subjects.

CONTROL TEST

1) The tested subjects' natural preference for cinnamon flavored food was evaluated by performing a single sample *t*-test to the *ProportionCCin* consumed by the control individuals, comparing it with randomness. Before the test, the normality of the response variable was verified using the Shapiro-Wilk test:

2.2 *ProportionCCin* = theoretical mean of 0.5

2) To subsequently understand if other measured variables could have influenced what individuals from the control test ate, we applied the following LMM model:

2.3 (Model 1): *ProportionCCin* = *SexInd* + *Day* + (1 | *Individual*),

where *SexInd* is a categorical variable and represents the sex of the control individuals (the tested subjects), having two conditions: "F" for females and "M" for males; *Day* represents the 3-day period of the preference test during which subjects and both food types were weighed; and *Individual* represents each tested subject for the control test.

As fixed effects we used *SexInd* and *Day*, without interaction (as we had no good reason to expect contrasting behaviors from males and females across days). As random effects we used *Individual* as a random intercept, due to the repeated measures that were done to the different tested subjects over the 3-day observation period.

HORIZONTAL TRANSMISSION EXPERIMENT

1) In order to evaluate whether the acquisition of preference for CCinnamon was related with the duration of the interaction, the observer's sex, the weight differences between individuals or the day of the preference test, the following statistical model was applied:

2.4 (Model 2): *ProportionCCin* = AverageIntTime * DifWeightDemObs * *SexObs* + *Day* +

+ (1 | *Individual*),

where *AverageIntTime* represents the Average Interaction Time, that is, the average duration of each nose-to-nose contact between observer and demonstrator individuals (in seconds) during the 30-minute interaction phase. Instead of *AverageIntTime*, other time variables could have been used, and hence we developed equivalent models where *AverageIntTime* was replaced for: *TotalIntTime* (the total duration of nose-to-nose contacts, in seconds); *NumInt* (total number of nose-to-nose contacts); and *MaxIntTime* (duration of the longest nose-to-nose contact, in seconds).

DifWeightDemObs represents the difference between the weight of each demonstrator-observer dyad; *Day* represents the 3-day period of the preference test during which subjects and both food types were weighed; and *Individual* represents each tested subject used in the horizontal transmission test; *SexObs* is a categorical variable and represents the sex of the observers (which was always the opposite sex of the demonstrators), having two conditions: "F" for females and "M" for males.

As fixed effects we used, *AverageIntTime* (or *TotalIntTime* or *NumInt* or *MaxIntTime*) in interaction with *DifWeightDemObs* and *SexObs*, plus *Day*. Like in Model 1, we used *Individual* as a random intercept. To determine which time variable best represents interaction time between dyads of observer and demonstrator individuals, we used the AIC procedure to select the best of the five models (Burnham and Anderson 2004).

2) Additionally, as another version of the same model, we used the variable *SameDem* instead of *SexObs*, representing the fact that each demonstrator was used with two different observers, on separate days. Since the sex of the observers was always the opposite of the demonstrators, the variable *SameDem* is dependent on the variable *SexObs*, and hence both variables could not be used together in the same model. Also, since *DifWeightDemObs* is between dyads of demonstrator-observer and the variable *SameDem* is also between dyads, but where a demonstrator is the same and the observers are different subjects, the interaction between these variables could not be included in the model.

2.5 (Model 3): *ProportionCCin* = AverageIntTime * DifWeightDemObs + *SameDem* + *Day* +

+ (1 | *Individual*),

Hence, here we used as fixed effects *AverageIntTime* or *TotalIntTime* or *NumInt* or *MaxIntTime* in interaction with *DifWeightDemObs*, plus *SameDem* and *Day*. Like in Models 1 and 2, we used *Individual* as a random intercept.

3) We also wanted to understand if there was a minimum interaction time required for an effective transmission of information about food. For that, we used the time variable *AverageIntTime* – the variable that performed best in Models 2 and 3 – to create another variable: the categorical variable *IntervalsTimeGroup*, with four classes of 3-second intervals each: 0-3s; 3-6s; 6-9s; >9s of interaction time. With this new variable, we developed the following LMM model:

2.6 (Model 4): *ProportionCCin = IntervalsTimeGroup * DifWeightDemObs* + (1 | *Individual*),

where *IntervalsTimeGroup* represents the new categorical variable; *DifWeightDemObs* represents the difference in weight between demonstrators and their observers, and was included here, in interaction with *IntervalsTimeGroup* because it had a significant effect in Models 2 and 3; and, like in the previous models, *Individual* represents a random intercept.

4) Finally, to compare the acquired preference for CCinnamon between the tested subjects in the Horizontal test and the Control test (to understand if social transmission was significantly important to the tested subjects acquisition of a food preference for CCinnamon), we applied a fourth LMM model:

2.7 (Model 5): *ProportionCCin = IntervalsTimeGroupAndControl* + (1 | *Individual*),

where the variable *IntervalsTimeGroupAndControl* is equivalent to the variable *IntervalsTimeGroup* from Model 4, but now including the control group. This analysis could not be done directly in Model 4, because the variable *DifWeightDemObs* cannot be tested with the control subjects (because in the control group there were no demonstrators). *IntervalsTimeGroupAndControl* is, therefore, a categorical variable with five classes: the control group and four classes of 3-second intervals each: 0-3s; 3-6s; 6-9s; >9s of interaction time. Like in the previous models, *Individual* represents a random intercept.

We then compared all the interval classes of interaction time among each other, using the *post-hoc* Tukey test. This was done to understand which classes were significantly different from each other and also to evaluate whether individuals that interacted for a shorter period of time were demonstrating similar food preferences to those of the control individuals (i.e., below a given interaction time threshold we expected observers not to be able to acquire any food related information from demonstrators and thus should not show a preference for the demonstrators' diet CCinnamon).

VERTICAL TRANSMISSION EXPERIMENT

1) To analyze if the 13 offspring born in the laboratory gained a preference for CCinnamon, which was the only food that their mothers ate during pregnancy and nursing (experiment 2), the following LMM model was applied:

2.8 (Model 6): *ProportionCCin = Treatment * SexInd + Day* + (1 | *Individual*),

where *Treatment*, a categorical variable with two conditions "Control" and "Horizontal", represents which test an individual had previously undergone. In this model we used the variable *Treatment* instead of the interaction time variables of the horizontal test, because we assume that the period of interaction between the offspring and their mothers was more than enough for the transmission of information to occur, without the need to distinguish individuals on the basis of interaction time.

SexInd, also a categorical variable, represents the sex of the individuals (the offspring and the controls), having two conditions: "F" for females and "M" for males; and *Day* represents the 3-day period of the preference test during which subjects and both food types were weighed. As fixed effects we used the variables *Treatment* and *SexInd* in interaction plus *Day*. As random effects we used, like in the previous models, *Individual* as a random intercept.

2) Also, we wanted to account for the fact that individuals from the same litter (siblings) were most probably similarly influenced by their mothers (*SameMother*) regarding the *ProportionCCin* they ate, but potentially not to those from other litters (and mothers). This had to be done in a separate statistical

model, because in the control group the variable *SameMother* did not exist. Hence, we applied the following LMM model:

2.9. (Model 7): *ProportionCCin = SameMother + SexOff + Day +*

+ (1 | Individual{SameMother}),

where *SameMother* represents individuals born in the same litter and hence from the same mother; *SexOff*, represents the sex of the offspring and the controls; *Day* represents the 3-day period during of the preference test which subjects and both food types were weighed, and *Individual* represents each test subject for the vertical transmission test. As fixed effects we used the variables *SameMother* plus *SexOff* and *Day*, with *SameMother* nested within *Individual*, and *Individual* as a random-intercept factor, resulting, like in the previous models, from the repeated measures that were taken from the different test subjects over the 3-day period.

LONG-TERM PREFERENCE TEST

We tested the long-term maintenance of the test subjects' preference for CCinnamon, from both vertical and horizontal social transmission experiments, by incorporating the *ProportionCCin* data from Day 30 in the variable *Experiment*:

2.10. *ProportionCCin = Experiment*

However, only individuals that were above a given interaction time threshold and showed a preference for CCinnamon were included in this analysis (those from the time classes, in the variable *IntervalsTimeGroup*, that represent a minimum interaction time between observers and demonstrators that allowed an effective information transmission about food). Since the test subjects from the Vertical Transmission experiment did not show a significant preference for CCinnamon, the long-term preference test was only applied to 12 subjects from the Horizontal Transmission Experiment. Hence, *Experiment* was a categorical variable with only three groups: Control, Horizontal and Long-term.

In this model, *ProportionCCin* was not normally distributed, so we performed a non-parametric Kruskal-Wallis test. It was expected that if a preference for CCinnamon was maintained 30 days after the social interaction occurred, significant differences would be found between the *ProportionCCin* of the Control and the Long-term groups but not between the Horizontal and Long-term groups, meaning that on average individuals from the Horizontal and Long-term groups ate significantly more CCinnamon than the Control individuals.

To compare the acquired preference for CCinnamon among the three conditions, we applied a Nemenyi *post-hoc* test with Tukey-distance approximation for independent samples.

TRANSMISSION EFFICIENCY

Lastly, we evaluated the success of horizontal information transmission in inducing food preferences to the Algerian mouse. Indeed, in order to apply any given methodology to further experiments and conservation programs, we should first be able to evaluate its success. As such, we evaluated the efficiency of information transmission by calculating, from our data, how many subjects need, in the future, to be captured and trained in the laboratory in order to obtain a given success rate of food preference transmission under the same methodology. For that, we calculated the percentage of individuals that acquired a preference for CCinnamon considering three preference levels: a) low preference, when observers ate only 60% of CCinnamon (*Eficiency60*); b) medium preference, when they ate more than 70% of CCinnamon (*Eficiency70*); and c) high preference, when observers ate more than 80% of CCinnamon (*Eficiency80*).

ETHICAL NOTE

This project was carried out taking into account the ethical guidelines stated in the "Guidelines for the treatment of animals in behavioural research and teaching" (Buchanan *et al*. 2012). The capture, transport and maintenance of animals was authorized by "Instituto de Conservação da Natureza e Florestas", the competent Portuguese authority, under the license number 528/2016/CAPT.

Young juveniles and females that were caught while pregnant (and could be immediately identified as such) were immediately released at capture location.

All remaining animals were taken to the laboratory and released in the same locations where they were captured at the end of each experiment. Animals born in the lab (from the vertical transmission experiment) were released as well. All animals were released taking into account a minimum distance between them to minimize their potential impact on established populations.

3. RESULTS

CONTROL TEST

1) When performing the single sample *t*-test to the control subjects, the proportion of CCinnamon (*ProportionCCin*) was not significantly different from randomness, i.e., from a theoretical mean of 0.5 preference (t (71) = -0.7020; p = 0.4850) (figure 3.1), indicating that *M. spretus* does not have a natural preference for either cinnamon or cocoa flavored chow.

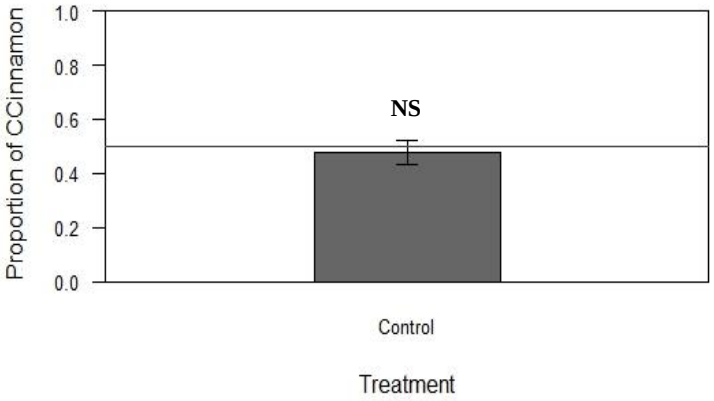

Figure 3.1 - Proportion of cinnamon flavored chow (CCinnamon) eaten by *Mus spretus* in the control test. *t*-test (71) = -0.7020; p = 0.4850). Error bars represent the standard error of the mean defined with the differences between individuals.

2) The best reduced LMM model, determining which explanatory variables were influencing the *ProportionCCin* eaten by the control subjects, excluded the variables *Day* (χ^2 (2) = 1.4075; p = 0.4947) and *SexInd* (χ^2 (1) = 0.3199; p = 0.5716). This means that the proportion of CCinnamon individuals ate was not significantly influenced over the 3-day period or by the sex of the individuals.

HORIZONTAL TRANSMISSION EXPERIMENT

Interaction Time Variable: *AverageIntTime*

1) In the analysis with *SexObs* (instead of *SameDem*), the triple interaction between *AverageIntTime*, *SexObs* and *DifWeightDemObs* (χ^2 (1) = 0.0315; p = 0.8592); the double interactions between *SexObs* and *AverageIntTime* (χ^2 (1) = 1.6438; p = 0.1998) and between *SexObs* and *DifWeightDemObs* (χ^2 (1) = 3.3013; p = 0.0692); and the variables *Day* (χ^2 (2) = 1.7362; p = 0.4197) and *SexObs* (χ^2 (1) = 0.0352; p = 0.8512), were all excluded. Thus, in the best reduced LMM model (with the AIC = 14.6120), only the interaction between *AverageIntTime* and *DifWeightDemObs* (F (1) = 11.7490; p = 0.0027) and the corresponding fixed effects (*AverageIntTime*: F (1) = 26.6460; p < 0.0001; *DifWeightDemObs*: F (1) = 10.2910; p = 0.0044) significantly influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test.

2) In the analysis with *SameDem* (instead of *SexObs*), the variable *Day* (χ^2 (2) = 1.8793; p = 0.3908), was excluded. Thus, in the best reduced LMM model (with the AIC = 14.1580), the interaction between *AverageIntTime* and *DifWeightDemObs* (F (1) = 7.8629; p = 0.0206) and the corresponding fixed effects (*AverageIntTime*: F (1) = 22.5120; p = 0.0011; *DifWeightDemObs*: F (1) = 8.1004; p = 0.0192)

significantly influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test; as opposed to the variable *SameDem* (F (11) = 1.3195; $p = 0.3441$).

Altogether, this means that (a) the proportion of CCinnamon that subjects ate was not different in the 3-day period tested; (b) there were no differences between the sexes of the observers (nor of the demonstrators, since it was always the opposite); (c) the use of the same demonstrator with two observers had no significant effect in the observers' response; and (d) the average interaction time between observer-demonstrator dyads varied across subjects, as well as the weight differences between demonstrators and observers, and additionally the two variables could be related, influencing the dependent variable (*ProportionCCin*).

Interaction Time Variable: *TotalIntTime*

1) In the analysis with *SexObs* (instead of *SameDem*), the triple interaction between *TotalIntTime*, *SexObs* and *DifWeightDemObs* (χ^2 (1) = 2.9052; $p = 0.0883$); the double interactions between *SexObs* and *TotalIntTime* (χ^2 (1) = 0.2686; $p = 0.6043$), between *SexObs* and *DifWeightDemObs* (χ^2 (1) = 2.7857; $p = 0.0951$), and between *TotalIntTime* and *DifWeightDemObs* (χ^2 (1) = 3.6127; $p = 0.0573$); and the variables *Day* (χ^2 (2) = 1.7362; $p = 0.4197$) and *SexObs* (χ^2 (1) = 0.0346; $p = 0.8524$) were all excluded. Thus, in the best reduced LMM model (with the AIC = 23.1960), only the variables *TotalIntTime* (F (1) = 18.4506; $p = 0.0003$) and *DifWeightDemObs* (F (1) = 5.7278; $p = 0.02612$), not in interaction, significantly influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test.

2) In the analysis with *SameDem* (instead of *SexObs* the variable *Day* (χ^2 (2) = 1.7362; $p = 0.4197$), was excluded. Thus, in the best reduced LMM model (with the AIC = 21.6320), the interaction between *TotalIntTime* and *DifWeightDemObs* (F (1) = 6.6528; $p = 0.0297$), as well as the fixed effect *TotalIntTime* (F (1) = 12.4794; $p = 0.0062$), significantly influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test; as opposed to the variables *SameDem* (F (11) = 1.2393; $p = 0.3797$) and *DifWeightDemObs* (F (1) = 4.9696; $p = 0.0528$)

Like in the previous model, this means that (a) the proportion of CCinnamon the subjects ate was not different in the 3-day period tested; (b) there were no differences between the sexes of the observers; (c) the use of the same demonstrator with two observers had no significant effect in the observers' response; and (d) the total interaction time varied across individuals as well as the weight difference between demonstrators and observers, and additionally the two variables could be related in the model with the *SameDem*, influencing the dependent variable.

Interaction Time Variable: *NumInt*

1) In the analysis with *SexObs* (instead of *SameDem*), the triple interaction between *NumInt*, *SexObs* and *DifWeightDemObs* (χ^2 (1) = 1.5903; $p = 0.2073$); the double interactions between *SexObs* and *NumInt* (χ^2 (1) < 0.0001; $p = 0.9867$), between *SexObs* and *DifWeightDemObs* (χ^2 (1) = 1.5367; $p = 0.2151$), and between *NumInt* and *DifWeightDemObs* (χ^2 (1) = 1.1998; $p = 0.2734$); and the variables *Day* (χ^2 (2) = 1.7362; $p = 0.4197$) and *SexObs* (χ^2 (1) = 1.8212; $p = 0.1772$) were all excluded. Thus, in the best reduced LMM model (with the AIC = 32.8000), only the variables *NumInt* (F (1) = 5.4402; p = 0.0297) and *DifWeightDemObs* (F (1) = 4.3259; $p = 0.0499$), not in interaction, significant influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test.

2) In the analysis with *SameDem* (instead of *SexObs*), the interaction between *NumInt* and *DifWeightDemObs* (χ^2 (1) = 0.9981; $p = 0.3178$); and the variables *Day* (χ^2 (2) = 1.7362; $p = 0.4197$)

and *SameDem* (χ^2 (11) = 17.7500; p = 0.0876), were excluded. Thus, in the best reduced LMM model (with the AIC = 32.8000), the variables *NumInt* (F (1) = 5.4402; p = 0.0297) and *DifWeightDemObs* (F (1) = 4.3259; p = 0.0499), not in interaction, significantly influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test.

Like in the previous models, this means that (a) the proportion of CCinnamon the subjects ate was not different in the 3-day period tested; (b) there were no differences between the sexes of the observers; (c) the use of the same demonstrator with two observers had no significant effect in the observers' response; and (d) the total interaction time varied across individuals as well as the weight difference between demonstrators and observers, influencing the dependent variable.

Interaction Time Variable: *MaxIntTime*

1) In the analysis with *SexObs* (instead of *SameDem*), the triple interaction between *MaxIntTime*, *SexObs* and *DifWeightDemObs* (χ^2 (1) = 0.9110; p = 0.3398); the double interactions between *SexObs* and *MaxIntTime* (χ^2 (1) = 0.3149; p = 0.5747), and between *MaxIntTime* and *DifWeightDemObs* (χ^2 (1) = 2.5488; p = 0.1104); and the variables *Day* (χ^2 (2) = 1.7362; p = 0.4197) and *SexObs* (χ^2 (1) = 0.0346; p = 0.8524) were all excluded. Thus, in the best reduced LMM model (with the AIC = 24.1960), the variables *MaxIntTime* (F (1) = 16.6382; p = 0.0006) and *DifWeightDemObs*: (F (1) = 8.0574; p = 0.011) significantly influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test; as opposed to the interaction between *SexObs* and *DifWeightDemObs* (F (1) = 4.1139; p = 0.0568) and the variable *SexObs* (F (1) = 0.5662; p = 0.4610).

2) In the analysis with *SameDem* (instead of *SexObs*), the variable *Day* (χ^2 (2) = 1.8138; p = 0.4038), was excluded. Thus, in the best reduced LMM model (with the AIC = 16.6790), the interaction between *MaxIntTime* and *DifWeightDemObs* (F (1) = 5.4322; p = 0.0447) and the corresponding fixed effects (*MaxIntTime*: F (1) = 20.4235; p = 0.0014; *DifWeightDemObs*: F (1) = 14.4440; p = 0.0042) significantly influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test; as opposed to the variable *SameDem* (F (11) = 1.9233; p = 0.1677).

Like in the previous models, this means that (a) the proportion of CCinnamon the subjects ate was not different in the 3-day period tested; (b) there were no differences between the sexes of the observers; (c) the use of the same demonstrator with two observers had no significant effect in the observers' response; and (d) the Maximum Interaction Time varied across individuals as well as the weight difference between demonstrators and observers, and additionally the two variables could be related in the model with the *SameDem*, influencing the dependent variable.

IntervalsTimeGroup

3) From the above results, we found that all the Interaction Time Variables had a significant effect in *ProportionCCin*. Therefore, since the four variables are not independent from each other, we chose to use the *AverageIntTime* to create the variable *IntervalsTimeGroup*, since *AverageIntTime* was the variable that best influenced its statistical models (with the smallest AIC values for both the models with *SexObs* and *SameDem*). We also included, in this analysis the variable *DifWeightDemObs*, because it was shown to have a significant effect in the model in interaction with *AverageIntTime*.

In the best reduced LMM model, the interaction between *IntervalsTimeGroup* and *DifWeightDemObs* (F (3) = 4.8999; p = 0.0132) and the corresponding fixed effect *IntervalsTimeGroup* (F (1) = 9.1116; p = 0.0009) significantly influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test; as opposed to the variable *DifWeightDemObs* (F (1) = 2.3855; p = 0.1420) (figure 3.2). The more time the subjects spent in each nose-to-nose contact, the more

CCinnamon they ate and this could be statistically related with the weight difference between demonstrators and observers.

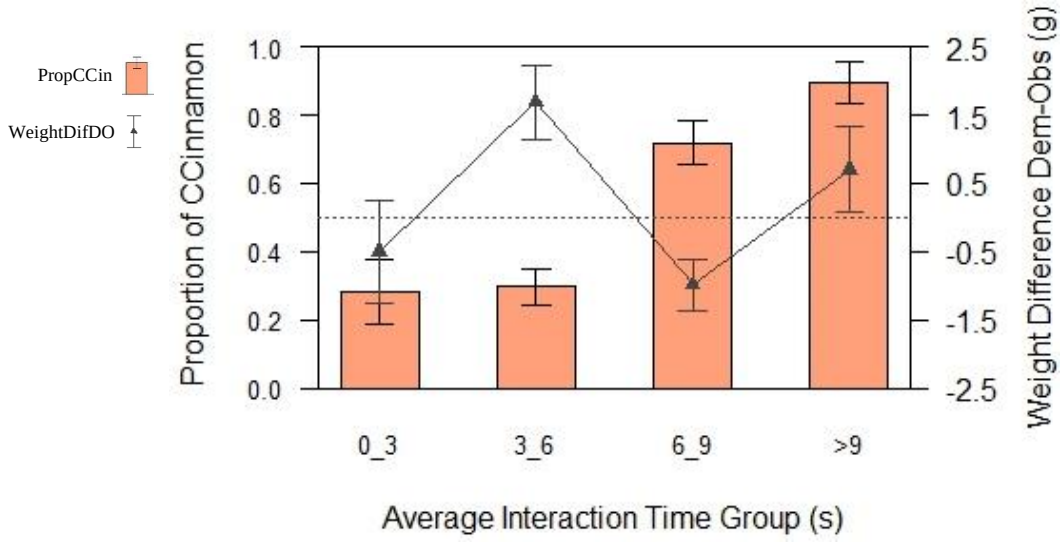

Figure 3.2 - Relation between *ProportionCCin, IntervalsTimeGroup* and *DifWeightDemObs*. Four time classes of 3-second intervals were defined, based on the *AverageIntTime*. Orange bars represent the proportion of CCinnamon eaten by *Mus spretus* individuals in the Horizontal Transmission Test: the more time the subjects spent in each nose-to-nose contact, the more CCinnamon they ate. The gray line represents the average weight differences between the demonstrator and the observer in each time interval class. Interaction between *IntervalsTimeGroup* and *DifWeightDemObs* significantly influenced the *ProportionCCin* ($F(3) = 4.8999$; $p = 0.0132$). Error bars represent the standard error of the mean defined with the differences between individuals.

IntervalsTimeGroupAndControl

4) Finally, to compare the acquired preference for CCinnamon between the test subjects in the Horizontal Transmission test and in the Control test, we used the categorical variable *IntervalsTimeGroupAndControl*. By applying the correspondent LMM model, the variable *IntervalsTimeGroupAndControl* ($F(4) = 7.1030$; $p = 0.0002$) also significantly influenced the *ProportionCCin* eaten by the tested subjects in the horizontal transmission test (figure 3.3).

In order to determine which time intervals where significantly different, we performed a Tukey test. Results showed that individuals that interacted less than 6s on average did not eat significantly different amounts of CCinnamon (0-3s *vs* 3-6s: $p = 0.9999$), nor did they eat more than the control individuals (Control *vs* 0-3s: $p = 0.1943$; Control *vs* 3-6s: $p = 0.0649$); on the contrary, they ate significantly less CCinnamon than the ones that had interactions longer than 6s on average (0-3s *vs* 6-9s: $p = 0.0002$; 0.3s *vs* >9s: $p = 0.0004$; 3-6s *vs* 6-9s: $p < 0.0001$; 3-6s vs >9s: $p = 0.0001$). Individuals that interacted for longer than 6s also ate significantly more CCinnamon than control individuals (Control *vs* 6-9s: $p = 0.0016$; Control *vs* >9s: $p = 0.0075$) (figure 3.3).

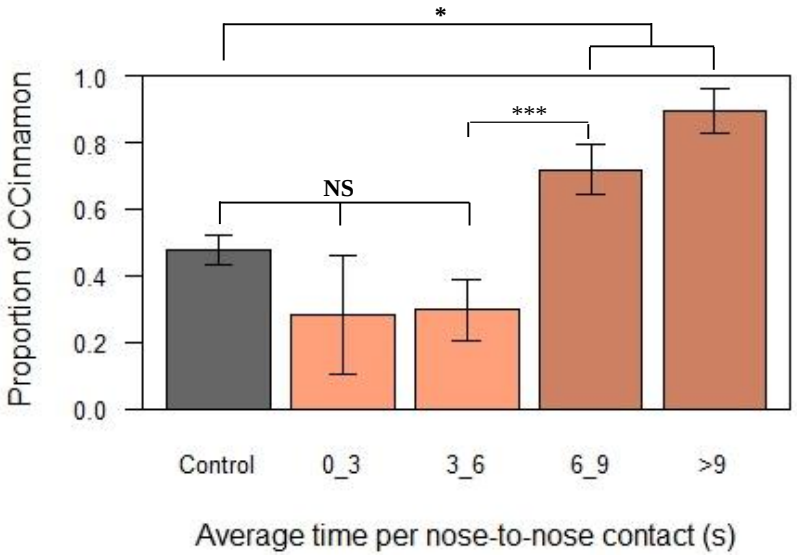

Figure 3.3 - Proportion of cinnamon flavored chow (CCinnamon) eaten for average social interaction duration between *Mus spretus* dyads. Four time intervals were considered (0-3s, 3-6s, 6-9s and >9s as in the *IntervalsTimeGroup*) plus the control group. Subjects that interacted less than 6s did not differ from the controls in the amount of CCinnamon eaten (Control *vs* 0-3s: $p = 0.1943$; Control *vs* 3-6s: $p = 0.0649$), while individuals that interacted more than 6s ate significantly more CCinnamon after the interaction (Control *vs* 6-9s: $p = 0.0016$; Control *vs* >9s: $p = 0.0075$). Error bars represent the standard error of the mean defined with the differences between individuals.

LONG-TERM PREFERENCE TEST

Only individuals that showed a significant preference from CCinnamon were included in this analysis (N=12), that is, only the individuals that interacted longer than 6s were considered. A significant effect between *Experiment* and *ProportionCCin* was found (χ^2 (2) = 17.0691; $p = 0.0002$). Individuals from the Horizontal test group ate significantly more CCinnamon than individuals from the Control group ($p = 0.0002$), but no more than those in the Long-term preference group ($p = 0.7489$). However, when compared to the control group, individuals from the Long-term Preference group did not eat significantly more CCinnamon ($p = 0.1816$). (figure 3.4).

This means that (a) the preference for CCinnamon was maintained across the 30 days for most of the tested subjects, though (b) there was a decrease in the amount of CCinnamon eaten by some of them (figure 3.4).

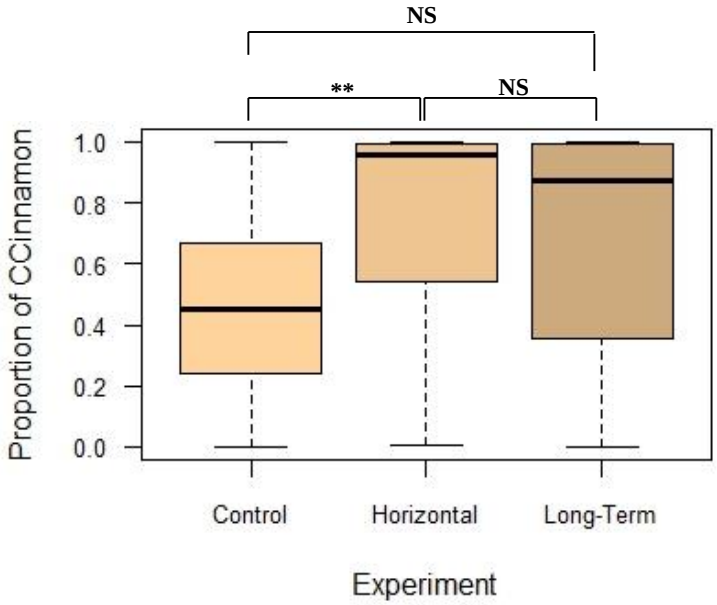

Figure 3.4 - Proportion of cinnamon flavored chow (CCinnamon) eaten by *Mus spretus* in the Control, Horizontal and Long-term preference tests. Individuals in the Horizontal test ate a significantly higher proportion of CCinnamon than the individuals from the control test (p=0.0002) but not from those in the Long-term preference tests 30 days later (p = 0.7489). The proportion of CCinnamon eaten in the Long-Term test was not significantly different from the Control test (p = 0.1816).

VERTICAL TRANSMISSION EXPERIMENT

Pilot test

Results from the vertical transmission pilot test show that the 4 offspring ate more cinnamon flavored chow – the chow their female progenitor ate during nursing – than the cocoa flavored chow. On average, over a 3-day period, the 3 female offspring ate 84%, 82% and 72% of cinnamon flavored chow and the male ate 64%. Thus, in total, the 4 offspring ate on average 76% of cinnamon flavored chow.

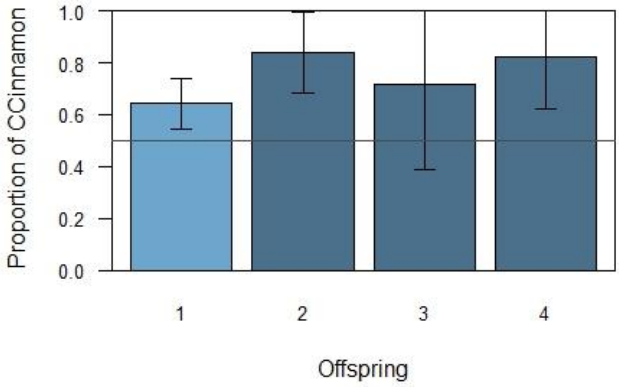

Figure 3.5 – Proportion of cinnamon flavored chow (CCinnamon) eaten by Mus spretus offspring in the vertical transmission pilot test. Proportion of cinnamon flavored chow (CCinnamon) eaten by each offspring over the 3-day period of the preference test (male offspring in light blue and female offspring in dark blue). Error bars represent the standard error of the mean defined with the differences between the three days tested. The horizontal line represents the 50% choice.

Vertical transmission test

1) In the final test, in determining the best reduced LMM model for the comparison between the vertical transmission test and the control test (Model 5), the interaction between *Treatment* and *SexOff* (χ^2 (1) = 0.0797; p = 0.7777) and the corresponding fixed effects (*SexObs*: χ^2 (1) = 0.2068; p = 0.6493;

and *Treatment*: χ^2 (1) = 2.0743; p = 0.1498), as well as the variable *Day* (χ^2 (2) = 0.1433; p = 0.9309), were all excluded.

This means that (a) the proportion of CCinnamon that was eaten by the test subjects was not significantly different between days; (b) there were no differences between the offspring sexes; and (c) there were no differences between the *ProportionCCin* that was consumed by the control individuals and the offspring (figure 3.6).

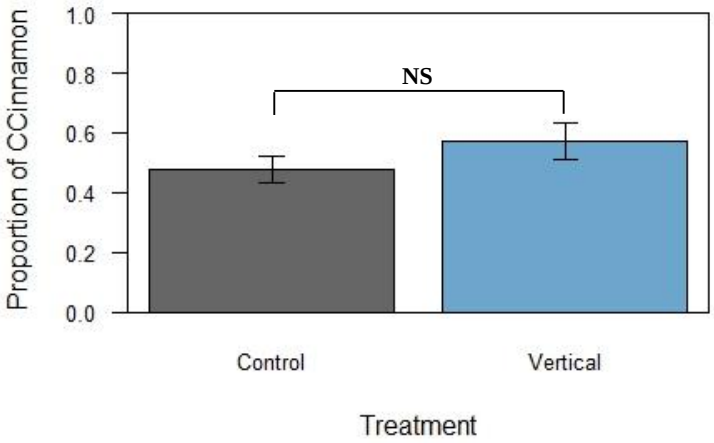

Figure 3.6 - Proportion of cinnamon flavored chow (CCinnamon) eaten by *Mus spretus* in the Control test *versus* Vertical transmission test. No significant difference was found between both conditions over a 3-day period (p = 0.1498). Error bars represent the standard error of the mean defined with the differences between individuals.

2) For the LMM model with the variable *SameMother*, this variable was excluded (χ^2 (3) = 1.2794; p = 0.7340), indicating that the females did no influence the *ProportionCCin* siblings ate (figure 3.7).

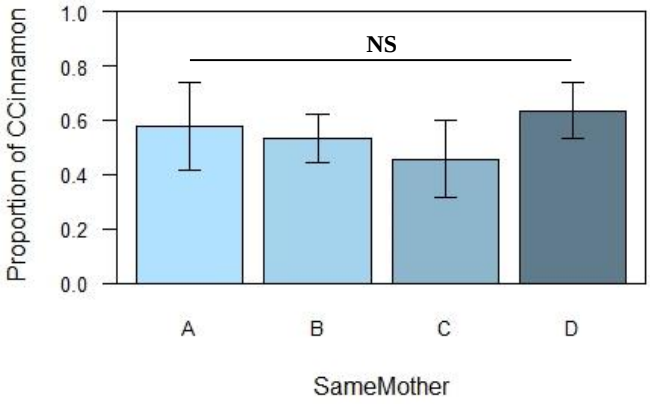

Figure 3.7 – Proportion of cinnamon flavored chow (CCinnamon) eaten by the *Mus spretus* offspring during the vertical transmission tests. Each bar represents the average *ProportionCCin* eaten by juveniles from a given litter over a 3-day period (litters: A=3, B=3, C=2, D=5 juveniles). There was no influence of the mothers in the proportion of CCinnamon eaten by the offspring (p = 0.7340). Error bars represent the standard error of the mean defined with the differences between individuals.

TRANSMISSION EFFICIENCY

Lastly, we calculated the success of the horizontal transmission method in inducing food preferences to the Algerian mouse by calculating Transmission efficiency. As such, according to the preference criteria adopted, we achieved different levels of efficiency, namely: 67%, if a low preference level (*Efficiency60*) is accepted; 64%, if a medium preference level (*Efficiency70*) is accepted; and 58% if a high preference level (*Efficiency80*) is accepted.

4. DISCUSSION

HORIZONTAL SOCIAL TRANSMISSION

Our main goal with this study was to understand if preference for a novel food can be socially transmitted and maintained between dyads of animals captured from the wild, similarly to what has been shown in laboratory rodents, and how we could use the knowledge gathered to propose its application to conservation management actions. We chose to work with the Algerian mouse, because we wanted to combine the use of wild animals from an abundant species and easy to live-trap, with a laboratory approach, as this constitutes the first necessary step for social learning research outside the laboratory (Kendal *et al*. 2010) and because social learning research is of outstanding importance to conservation (Blanchet *et al*. 2010). Also, since *M. spretus* is a close relative of the house mouse, a species in which social transmission of information about food has been shown to occur with laboratory animals (Valsecchi and Galef 1989), it should be especially interesting to compare our results with those ones. Would Algerian mice, caught from the wild, with unknown social backgrounds and potential behavioral constraints to the full transmission of social information (age, dominance status, etc.), be also influenced as the laboratorial house mouse by what its conspecifics eat, when confronted with novel foods?

The transmission of social information should be favored when the stimulus is new or uncertain (Valone and Templeton 2002, Laland 2004; Wagner and Danchin 2010), so when deciding on whether to eat a novel food, individuals should rely on food cues inadvertently provided by healthy conspecifics and eat what those conspecifics had already eaten. Indeed, we found that naïve (observer) individuals that interacted long enough with experienced (demonstrator) individuals preferred the novel food that the demonstrators ate, which was cinnamon flavored chow, instead of cocoa flavored chow. Hence, as the duration of nose-to-nose interactions between dyads of observer-demonstrator individuals increased, so did the proportion of cinnamon flavored chow eaten by the observers. On the contrary, when the interaction was short (less than six seconds per contact), observers did not acquire a preference for any given chow and ate similar amounts of both flavored chows, similarly to the control individuals. Our results thus clearly show that horizontal transmission of information occurs in wild Algerian mice, similarly to what has been described for the laboratory house mouse. However, a minimum interaction time per nose-to-nose contact (≈6s) is needed for the information transfer to successfully occur in *M. spretus*. Similar behavioral patterns were found for the total duration and total number of nose-to-nose contacts, as well as for the duration of the longest nose-to-nose contact, further supporting the idea that the duration and frequency of the interaction between observers and demonstrators is very important for the transmission of food preference to occur.

To our knowledge, this minimum interaction time has not been measured in studies with laboratory rats and house mice (Galef and Wigmore 1983; Valsecchi and Galef 1989). Given the strong influence it had in the transmission of information between *M. spretus* individuals, interaction time should clearly be a factor to have into account when promoting social learning. Its especial high relevance in our study could be related with the fact that, contrary to mouse and rat studies, individuals were not allowed to familiarize with each other before the social interaction – which we had to avoid because we tested male-female dyads and did not want them to mate. *M. spretus* individuals met for the first time in the social interaction phase of the experimental protocol and this may have decreased the average duration of interactions, as individuals were probably more cautious and less prone to close contact with conspecifics. Additionally or alternatively, our results also suggest that wild animals could be less social than laboratorial animals, constituting a behavioral constraint to the full transmission of social information in the wild. This hypothesis should be tested in the future, both to improve our understanding of social information transmission in the wild, as well as well to improve its

implementation in conservation actions. Moreover, our study not only highlights the importance of maximizing interaction time variables in protocols with observer-demonstrator interactions, but also the importance of including these variables in the statistical models used to evaluate the success of the methodology.

We also found an effect of the difference in weight between demonstrators and observers, including a significant statistical interaction between such weight differences and nose-to-nose interaction times. However, by carefully observing figure 3.2, we can conclude, from a biological point of view, that this effect may be a statistical artifact, having little importance. It is due to the fact that individuals from the 3-6s interaction time class had the demonstrator smaller than the observer and the individuals from the 6-9s class had the opposite. Yet, in the other two classes (0-3s and >9s) the weight differences were closer to zero. Hence, the data show no clear relation (for all time classes) between higher weight differences in demonstrator-observer dyads and shorter interaction times and between smaller weight differences in demonstrator-observer dyads and longer interaction times. This means that we cannot attribute the differences in interaction time to the weight differences found between demonstrator-observer dyads. Differences in interaction time could be attributed to several factors namely, social status, health condition, age, to name a few.

A much simpler explanation is available: because individuals were paired randomly, the weight differences between observers and demonstrators were probably due to the simple fact that males were significantly bigger than females, as the Mann-Whitney analysis comparing the weights between females and males – that we did *a posteriori* – supports ($W = 6867$; $p = 0.0153$).

Although we limited this study to male-female interactions because, as we explained before, these are considered to be the most common interactions in nature, it would be interesting to perform this experiment using only females (female-female dyads). Although their vital areas are smaller than those of males (Hurst *et al*. 1996; 1997), females share territories and often contact among them, and as such the probability of social transmission to occur between females should also be high. Also, to apply this method in the field, the more animals we can use to transmit the information, the better. If social information is equally transmitted between females, we would no longer be limited to staging opposite sex interactions and thus could more easily increase the number of individuals used, and ultimately the protocol's efficiency. Additionally, statistical and potentially behavioral effects of weight differences between observers and demonstrators would also be avoided.

VERTICAL SOCIAL TRANSMISSION

Given our finding that horizontal transmission of food preferences occurs in *M. spretus*, we decided to evaluate whether it also occurred between a mother and its offspring – vertical social transmission.

The pilot test – performed when a pregnant female was captured unintentionally – suggested that juveniles acquired a preference for the food their mothers ate during the late stage of the pregnancy and nursing – the four offspring ate on average 76% of cinnamon flavored chow over a 3-day period, an amount considerably higher than what expected by chance. Yet, when a formal experiment was carried out – the vertical transmission test, covering the entire pregnancy and nursing – the offspring of the four females used as demonstrators did not eat significantly more cinnamon flavored chow than the controls. Nevertheless, given the small sample size (4 females, 13 offspring) it is not possible to draw any definitive or strong conclusions.

Although in the beginning of the field season females were almost as common as males during capture sessions, as the seasons progressed (mainly from March to July, in spring/summer) females were trapped more sporadically (females were probably nursing the pups), and the ones captured were frequently pregnant, which implied their immediate release. Warmer conditions also decreased mice capture success and as such we were unable to capture any additional females for the vertical transmission test. Further studies using the same methodology and additional animals should then help clarifying if vertical social transmission does indeed occur in the Algerian mouse as it was demonstrated for the laboratory rat (Hepper 1988; Galef 1977; Galef and Henderson 1972; Martin and Alberts 1979; Galef 1971, 1981; Galef and Clark 1971a,b).

But supposing that a similar trend is still obtained using a larger sample size, one possible explanation for the lack of social transmission of food cues between mother and offspring could be the fact that *M. spretus* is a generalist species, and as such it might have a natural tendency to taste new foods independently of the stimuli provided by mothers during pregnancy and nursing. This hypothesis suggests that the use of social information to select food may not be a strategy present in the early life stages of the Algerian mouse, to whom a social learning propensity might only develop in older juveniles or even during adulthood. A mechanism of this type has been shown in rat pups (see a review in Ralphs and Provenza 1999) where, through individual food aversion conditioning (and not social learning), juveniles formed weaker aversion than adults and were much more prone to try different foods than adults. These findings are interesting, but they contrast with the studies showing the existence of vertical transmission of food preferences by social learning also in rats (Hepper 1988; Galef 1977; Galef and Henderson 1972; Martin and Alberts 1979; Galef 1971, 1981; Galef and Clark 1971a,b), suggesting that individual learning by food aversion conditioning is a totally different mechanism from social learning. Using other stimulus could also be an alternative; despite being an effective stimulus in horizontal transmission, cinnamon might not be suitable to test the transmission of food cues through the placenta or milk (eg: being available in insufficient levels for the transmission to occur). None of these hypotheses is, however, mutually exclusive and only further studies using a larger sample size will help disentangling between the vertical and the horizontal (i.e., only-between-adults) transmission hypotheses.

Vertical social transmission is the strongest vector of information inheritance across generations (Wagner and Danchin 2010; Danchin *et al*. 2011), making vertical transmission experiments of extreme importance when applied to conservation actions, at least if long term influences – feeding habits in this case – of a wild population are needed.

LONG-TERM MAINTENANCE OF AN ACQUIRED PREFERENCE

To propose the application of social transmission of food preferences as a conservation tool to be applied in the field, it is important to understand not only if and how information is transmitted, but also whether the acquired preference is maintained over time.

Our results, using only individuals that had acquired a preference during the short time period of the horizontal transmission test (N=12), show that individuals maintained the acquired preference after a 30-day period without contact with cinnamon flavored chow, since no differences were found between the proportion of cinnamon flavored food eaten in the horizontal test and that eaten after 30 days (long-term preference test). This is especially interesting as it shows that wild *M. spretus* individuals were able to acquire a food preference through social transmission, and were additionally able to preserve it for at least 30 days without further contact with the chow, under laboratory conditions.

Maintenance of an acquired preference over a long time period (30 days) has already been shown in laboratory rats (Galef and Whiskin 2010). In our study, Algerian wild mice were additionally allowed the opportunity to eat the novel food during the 3-day period following the social interaction which, theoretically, should have allowed *M. spretus* individuals to form an even stronger memory of the cinnamon flavored chow. However, this was not the case, because the proportion of cinnamon flavored food eaten in the long-term preference test was also not significantly different from that eaten in the control test. This suggests that besides maintaining an overall preference for cinnamon, there was at the same time a decrease in preference through time. Although the median is clearly elevated (87.13%), the first quartile is in the 35.34% of cinnamon eaten, equivalent to the median of the control, meaning that some individuals ate from both foods equally – while the majority still preferred cinnamon. What this suggests is that frequent social interactions are probably needed to maintain a strong food preference, as well as recurrent contact with the stimulus that is being transmitted. This can probably be due to the fact that, since *M. spretus* is a generalist, its diet basically reflects the availability of resources, probably not depending so much in long-term memory processes for finding food (Palomo *et al*. 2009).

This result also makes us suggest, one more time, that the interaction time between observers and demonstrators might not have been ideal, even for those individuals that interacted, on average, for more than six seconds each time. Perhaps longer mean interactions times, or more times interacting for more than six seconds would have been necessary for longer memorization. On the other hand, since we used the same chow with cinnamon or cocoa powder, the common odor of the chow could have influenced the results, making it difficult for individuals to distinguish between cinnamon and cocoa odors.

In future studies it would be wise to use more natural foods (like grass seeds or fruits) instead of chow, as this will help us to better understand how social transmission of food preferences work with foods that individuals already tasted before being socially conditioned, in a next step for implementing these studies in their natural environments. Further studies using wild species both in the laboratory and *in situ* are necessary to fully understand the cognitive processes involved in social transmission of information.

EFFICIENCY OF SOCIALLY TRANSMITTED FOOD PREFERENCES AND IMPROVEMENTS

If we wish to implement the methodology used as a protocol to be applied in future studies – namely conservation studies, where we wish to successfully condition animals in the laboratory to certain foods, and to subsequently use those animals as demonstrators in the wild, to induce the transmission of social food preferences to the entire population – it would be useful to determine its efficiency. That is, it would be useful to know how many animals will successfully acquire a food preference via social learning. Here we considered three levels of food preference: a) low preference, when observers ate only 60% of cinnamon flavored food; b) medium, when they ate more than 70%; and c) high, when observers ate more than 80%.

In our study, more than 50% of the individuals acquired a food preference for cinnamon through social interaction with their conspecifics in the three preference levels considered. In other words, independently of how stringent is the preference criteria used, more than 50% of the individuals always acquired a preference after the social interaction. In fact, even when the strictest preference criteria is used and individuals are required to eat more than 80% of cinnamon flavored chow for it to be considered as preference, still 58% of individuals fall into that category. Although we aim for the highest efficiency possible, either of the efficiency values obtained should be considered a good result, especially since we used wild animals, with unknown individual histories that can potentially affect the transmission of social preferences.

CASE-STUDY AS A MANAGEMENT TOOL FOR CONSERVATION BIOLOGY

The anthropological impact on ecosystems has increased exponentially over the years, making the conservation of species and their natural habitats more challenging every day. Hence, it is of major importance to implement new methodologies that can quickly change the course of events (Whitehead 2010; Kendal *et al.* 2010). A problem of many conservation and management actions is that they have limited effect over time (a few generations only) and its implementation often takes too long before becoming efficient (Whitehead 2010). By using the transmission of social information and social learning as a conservation tool, we will be taking advantage of the fact that a behavior will spread between individuals in a population and across generations faster than by genetic inheritance, saving time in the adaptation process, which could be crucial to critically endangered species (Richardson and Boyd 2005).

Therefore, idealizing the best way to manage endangered species in the wild can have a key role in the success of conservation actions. Since an animal's feeding habits are one of the most vital components for the survival of a species, developing the best methodologies to help solve foraging and food choice associated problems is essential. Hence, here we present some suggestions where we believe the results obtained in this study could be applied:

1. When there is a dangerous/poisonous invasive species that is killing a local predator, if instead of trying to eradicate the invasive species (unrealistic in most instances or very costly, as in the case of the Kakapo Recovery Plan in New Zealand; Lloyd and Powlesland 1994), or having to teach all animals to avoid eating harmful food (as in the examples of the northern quoll, the Australian carnivorous marsupial, O'Donnel *et al*. 2010; and the Australian bluetongue lizard, Price-Rees *et al*. 2011), researchers could use social information transmission tools, making their task much easier. This is because only a few animals need to be captured, brought to the laboratory and taught to prefer or avoid a given food, which, after being released again in the wild, will spread the information in the population by cultural diffusion (as it has already started to naturally happen in northern quolls, because non-conditioned offspring of previously conditioned mothers are learning from their mothers to avoid eating the dangerous prey, the cane toad; Webb *et al*. 2015).
2. When a species' food is becoming extinct, inducing a change in a population's feeding habits by increasing its preference for a less preferred or new (and harmless) prey could be an easier and faster strategy to implement than relocating the species or teaching each animal individually (Gustavson and Gustavson 1985; Lloyd and Powlesland 1994). However, because changing a species feeding habits can be ethically contested and dangerous, this should only be used in extreme cases where there is no other better/realistic alternative and always taking into account the ecological implications, trying to balance the risks and the benefits.
3. When animals represent a plague (e.g. in agrosystems), social learning could be used to condition a preference for another food. In some cases, the use of poisonous and killing traps could potentially be minimized. This is for example the case of Guinea-Bissau chimpanzees, that are being killed for attacking agricultural fields on which local people rely on to survive (Carvalho *et al*. 2014). Inducing a change in their current feeding habits, so that they return to feed on their natural past food diet, through social learning could potentially decrease the amount of food taken from agricultural fields and help locals and chimpanzees to live together more peacefully.

In all these cases, training animals to socially transmit an acquired behavior or preference (as Aplin *et al*. 2015 did with great tits using puzzle feeders) might represent a reduction in terms of costs associated with conservation actions, and in terms of recovery time for many species. Given the

exponential progression of social learning through a population, only a few individuals have to be conditioned to change their feeding habits for it to rapidly spread in the population (Kendal *et al*. 2010; Whitehead 2010; Aplin *et al*. 2015).

We strongly believe that the successful transmission of food preferences in wild *M. spretus* should serve as an indication that this approach can be taken outside the laboratory, which, if successful, could be transformed in a management tool in the protection and recovery of endangered species that use social transmission to learn about food preferences.

Nevertheless, and although social transmission might seem a good management tool, it is imperative to always carefully evaluate the ecological implications of influencing and potentially changing an individual's or population feeding habits, always balancing the risks and the benefits. When erroneous food preferences are implemented by social learning, and become culturally conserved in a population across generations, they can become maladaptive or an ecological disaster (Whitehead 2010). Moreover, diverse behavioral strategies, and hence not very strong or definitive food preferences, are also crucial to an individual's fitness, often being the way through which animals interact and plastically adapt to their abiotic and biotic environment (Sutherland 1998).

5. FINAL REMARKS

Taken altogether, the results of these experiments have allowed us to reach some interesting conclusions: (1) wild male and female Algerian mice can socially transmit food preferences horizontally (i.e., between conspecifics from the same age class or generation); and (2) the acquired food preference was maintained for a period of at least 30 days without contact with the new food. Nonetheless, (3) there was a slight decrease in preference over time, suggesting that additional interactions, as well as regular presence of the stimulus, might be needed to maintain a strong food preference. Finally, (4) given the low sample size, it was not possible to ascertain whether vertical transmission between mother and offspring occurs in *M. spretus*. Further studies using a larger sample size should easily clarify this.

Our results are quite satisfactory, but could be improved if in future studies we use female-female interactions, assuming that females will interact with each other for longer periods of time. Alternatively, or additionally, we propose that in future studies the duration of the interaction phase of the experimental setup should be increased to maximize the number and duration of interactions between individuals (both in male-female and female-female dyads), allowing more time to the individuals to habituate to one another, as well as to the enclosure, and thus potentially increase the success of the methodology.

We hope our results will help narrowing the gap between Conservation actions and Animal Behavior research, highlighting the importance of applying similar methodologies to real case scenarios, through the creation of additional methodologies and management tools, both in the laboratory and *in situ*, that could help in the recovery of endangered social species.

Lastly, ethical considerations should always be taken into account and further experiments, namely on the long-term prevalence (across many generations) of the acquired social influences, are necessary. Each species own specificities should always be taken into consideration before applying any new methodology, balancing its risks and benefits.

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